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DECONTAMINATION OF HEAVY METALS FROM DEWATERED SLUDGE BY *Acidithiobacillus ferrooxidans*

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Abstract : Bioleaching process using *Acidithiobacillus ferrooxidans* for the removal of heavy metals from dewatered municipal sewage sludge was investigated. Sequential extraction results showed that the heavy metals (Cd, Cu, Ni, Pb and Zn) were associated with exchangeable and carbonate fractions in the dewatered sludge. Short-term (4 days) bioleaching experiments for Zn, Cu and Cd were performed under various process conditions such as initial concentrations of Fe^{2+}, sulfur addition and input amount and particle size of the sludge. When lower content of the sludge was added, higher amounts of the metals were extracted into solution. However, higher initial concentration of Fe^{2+}, sulfur addition and fine particle size of the sludge did not facilitate the metal extraction during a period of 4 days. Long-term (40 days) leaching study indicated that biolixiviation using this acidophile can be effectively employed in the process of decontamination of heavy metal-laden dewatered sludge.

Key Words : *Acidithiobacillus ferrooxidans*, bioleaching, dewatered sludge, heavy metals

INTRODUCTION

As a result of population increase and economic growth, wastewater containing high amounts of heavy metals has originated from household wastes as well as many metal treating industries. Especially in the case of household wastewater, various metal-laden effluents are subject to be discharged to sewage systems without proper treatment. Such wastewater with high concentrations of toxic heavy metals leads to the production of the sewage sludge contaminated with the metals of environmental concern.

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Biological wastewater treatment techniques have been conducted for various purposes such as organic matter removal, nitrification and solid separation. However, electrostatic interaction of cationic heavy metals with anionic ligands on bacteria and biopolymers (e.g., proteins, polysaccharides, lipids, lipoproteins, nucleic acids, and glycocalyxes) generally causes biological wastewater treatment system to produce the sludge containing high concentrations of heavy metals.[1,2)]

Treatment and disposal of the wastewater sludges become a major environmental problem since more intensive treatment methods should be applied to the sludge than wastewater itself. Sludge disposal methods currently in use are landfill, incineration, ocean dumping and agricultural land application. Among these, the land application is the most attractively employed method since dewatered sludge can serve as a valuable resource of fertilizer or soil conditioner which facilitates nutrient transport and increases water retention.[1] However, before agricultural application of the dewatered sewage sludge, it is necessary to give full consideration on the levels and kinds of priority pollutants in the sludge. In addition to removal of pollutants such as PCBs and pathogens, a critical step in the decontamination of the dewatered sludge is to remove toxic heavy metals since heavy metals are not degradable biologically and physicochemically and thus, once released into the soil environments, they have high potential to deteriorate soil quality and groundwater supply and hence human health and safety.[3]

Conventional physicochemical treatment methods to remove heavy metals from the dewatered sludge are sometimes uneconomical or ineffective due to high cost of installation and operation, production of hazardous byproducts and unsatisfactory metal solubilization. In such a sense, biological leaching can be considered as an alternative because of many advantages such as high efficiency of metal removal and low operational cost compared to the other methods.[4]

Bioleaching is a process during which metals are dissolved from such solid matrix as the dewatered sludge by microorganisms including *Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *At. thermophilica*. Among these microorganisms, *At. ferrooxidans*, previously named as *Thiobacillus ferrooxidans*, has been widely used as an efficient leaching mediator in several commercial bioleaching facilities of, for example, metal recovery from low-graded ores.[5] This acidophile, iron-oxidizing chemoautotroph acts as a catalyzer to enhance direct dissolution of minerals[6] and/or indirect oxidation of sulfides by bacterially produced Fe^{3+}[7] during which changes in oxidation state of Fe result in increase in metal mobility. In addition, the bacterium can maintain significantly low pH of solution for a long duration; this is notably important in metal leaching processes since generally, the lower are pH values, the higher are extracted metal amounts. To achieve sufficiently low pH for a long period, active metabolism of this chemoautotroph should be attained and, therefore, proper amount of Fe^{2+} must be present in the solution or materials to be treated.

The objective of this research was to evaluate the efficiency of bioleaching for decontamination of heavy metal-laden dewatered sludges before land application. Physicochemical characteristics of the sludge including mode of heavy metal occurrence were examined. Both short-term (4 days) and long-term (40 days) batch bioleaching tests were conducted under various experimental conditions and the efficiencies were compared.

MATERIALS AND METHODS

Characterization of the Dewatered Sludge

Dewatered sludge samples were obtained from a municipal sewage treatment plant in Gwangju and stored at 4°C in refrigerating polyethylene bags. The values of pH were separately measured in supernatant of the sludge-distilled water mixture and the sludge-dilute solution of $CaCl_2$ mixture.[8] Water content, organic matter content and specific gravity of the sludge were measured according to APHA *et al.*[9] Total Cd, Cu, Ni, Pb and Zn in the dewatered sludge were determined after acid digestion following U.S. EPA method 3050B.[10]

To investigate heavy metal partitioning into several phases constituting the dewatered sludge, a sequential chemical extraction method was carried out using a series of selective extractants. The procedure employed in this technique was designed to allow metals associated with exchangeable (Step I), carbonates (Step II), Fe/Mn oxides (Step III), organic (Step IV) and residual fractions (Step V) to be determined. The operational conditions of each extractant were in depth represented by Jang.[11)]

Bioleaching Experiments

Short-Term Bioleaching : The inoculum for bioleaching experiments was prepared in a 300 mL Erlenmeyer flask containing 150 mL 9 K medium (Table 1)[12)] supplemented with 3% (w/v) dewatered sludge. The medium pH was adjusted to 2.0~2.2 with 5 M H_2SO_4 and the flask was agitated at 200 rpm in a shaking incubator at 30°C. When most Fe^{2+} was observed to be oxidized to Fe^{3+}, 25 mL of the medium was replaced with same volume of fresh medium. This process permitted a continuous growth of the cells. After nearly 30-day adaptation period to the sludge, 10% (v/v) of *At. ferrooxidans* culture solution was used as an inoculum for the following batch experiments. In the batch experiments, the initial pH of the medium was also adjusted to 2.0~2.2 using 5 M H_2SO_4.

To investigate the relationship between Fe^{2+} oxidation and cell growth and its effects on pH and oxidation-reduction potential (ORP) of the solution, 150 mL 9 K medium with 3% (w/v) dewatered sludge was inoculated with the sludge-adapted cells. After 3 mL samples were withdrawn from the batch flask at every 24-hour interval, solution pH, ORP, cell number and the concentration of Fe^{2+} were measured.

The effects of experimental conditions such as total input amount (1, 4, 10 and 15% (w/v)) and size (> 2 mm and < 2 mm diameter) of the sludge, initial concentration of Fe^{2+} (0, 4, 9, 15 and 20 g/L) and addition of sulfur (0, 1, 3 and 5% (w/v)) on the extent of heavy metal extraction during sludge bioleaching were investigated. To do this, after 4-day leaching period, samples were centrifuged at 10,000 rpm for 10 minutes to separate solid and liquid and then supernatants were passed through a 0.2 μm-pore membrane filter (Adventec MFS, USA) to guarantee particle free solutions. Dissolved Zn, Cu, and Cd were determined.

Table 1. Composition of the 9 K medium for the cultivation of *At. ferrooxidans*

Solution A (in 700 mL distilled water)		Solution B (in 300 mL distilled water)	
Components	Amounts	Components	Amounts
$(NH_4)_2SO_4$	3.0 g	$FeSO_4 \cdot 7H_2O$	45 g
KCl	0.1 g		
K_2HPO_4	0.5 g		
$MgSO_4 \cdot 7H_2O$	0.5 g		
$Ca(NO_3)_2 \cdot 4H_2O$	0.014 g		

Long-Term Bioleaching : An air-diffused, stirred reactor (4 L) was charged with 2.5 L of 9 K medium with 20 g/L of Fe^{2+}, 30% (v/v) of sludge-adapted cell culture solution and 3% (v/v) of ground dewatered sludge (< 2 mm diameter). The temperature of the reactor was maintained at 30°C by a heating-cooling water circulator. The medium pH was initially adjusted as 2.0 by addition of 5 M H_2SO_4. Ten-mL samples were removed from the reactor at regular interval (every 10 day) over a leaching period (40 days). The procedure for separating liquid from solid was same as previously described and Zn, Cu and Cd were determined. Changes in morphology of the sludge particles were examined through scanning electron microscopic (SEM) observation.

Analytical Methods

Oxidation-reduction potential and pH were monitored using each electrode connected to a pH-ORP meter (Orion model 250A, USA). The concentration of Fe^{2+} was measured using a UV-vis spectrometer (Perkin Elmer Lambda 12, USA) at 510 nm after mixing with *o*-phenanthroline. Ferric ion content was calculated from total Fe, which was determined using atomic absorption spectrophotometer (AAS; Perkin Elmer 5100PC, USA), subtracted by measured Fe^{2+}. Direct cell counting was performed through a Nikon Microphot-SA microscope (Japan). Cadmium, Cu, Ni, Pb, and Zn were determined by AAS.

RESULTS AND DISCUSSION

Characteristics of the Dewatered Sludge

The physicochemical properties and concentrations of heavy metals (Cd, Cu, Ni, Pb, and Zn) in the dewatered sludge are shown in Table 2. Organic matter content in the dewatered sludge (42.5%) showed that the sludge contained more amounts of organics than normal soil types (average organic matter content of 7.7% for alluvial, high land, and garden soils in Korea[13)]). Such high organic matter content in the dewatered sludge can likely provide favorable conditions for plant growth after agricultural application of the sludge. As seen in Table 2, the total concentrations of heavy metals in the dewatered sludge were high, showing the order of Zn > Pb ≥ Cu > Ni > Cd.

Results of the sequential extraction for Cd, Cu, Ni, Pb, and Zn in the dewatered sludge are represented in Figure 1. A large fraction of metals was closely associated with weak acid-soluble (Step II) or even with water-soluble phase (Step I); i.e., readily leachable under normal environmental conditions. In the cases of, especially, Ni and Zn, portions of Step I plus II were observed to be significantly high. After agricultural application, the metals incorporated with such phases can be easily leached out into pore water and groundwater from the sludge due to a slight change in soil conditions. Before applying the sludge for agricultural purpose, it is needed to consider the alteration of mobility and bioavailability of toxic metals in the dewatered sludge in response to environmental change as well as the total concentrations of the heavy metals.

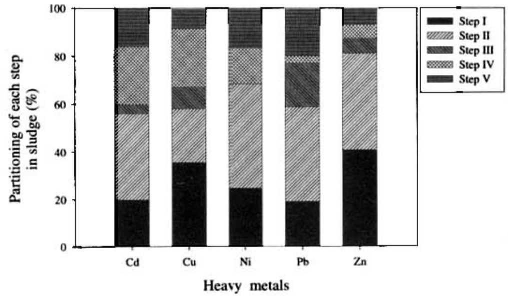

Figure 1. Mode of heavy metal occurrence in the dewatered sludge determined by sequential extraction method (Step I: exchangeable, Step II: carbonates, Step III: Fe/Mn oxides, Step IV: organic and Step V: residual fraction).

Short-term Bioleaching of the Dewatered Sludge

As described earlier, *At. ferrooxidans* obtains energy from oxidizing Fe^{2+} or reduced forms of sulfur to Fe^{3+} or SO_4^{2-}, respectively (e.g., Eq. (1)).

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4 \quad (1)$$

Relationships between pH, ORP, concentrations of Fe^{2+} and Fe^{3+}, and cell growth are shown in Figure 2. Direct proportion of Fe^{2+} and inverse proportion of Fe^{3+} with cell growth indicated that the bacteria clearly oxidized Fe^{2+} to Fe^{3+} as an energy source to support the growth and maintenance. The values of pH and ORP over the period of exponential cell growth were 2.0～2.5 and 500～600 mV, respectively.

In order to investigate the capacity of *At. ferrooxidans* for producing Fe^{3+} from Fe^{2+} oxidation, the production of biotic and abiotic Fe^{3+} were compared (Figure 3). The concen-

Table 2. The physicochemical properties and heavy metal concentrations of the dewatered sludge

pH		Water content (%)	Organic matter content (%)	Specific gravity	Concentrations of heavy metals (mg/kg)				
Distilled water	0.01 M $CaCl_2$				Cd	Cu	Ni	Pb	Zn
8.1±0.1	7.8±0.2	24.9±0.7	42.5±8.7	1.16±0.06	13±3	778±29	69±12	791±35	1,090±3

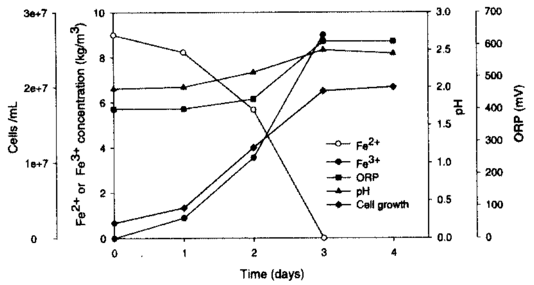

Figure 2. Relationships between pH, ORP, concentrations of Fe^{2+} and Fe^{3+} and cell growth during 4 days of cultivation.

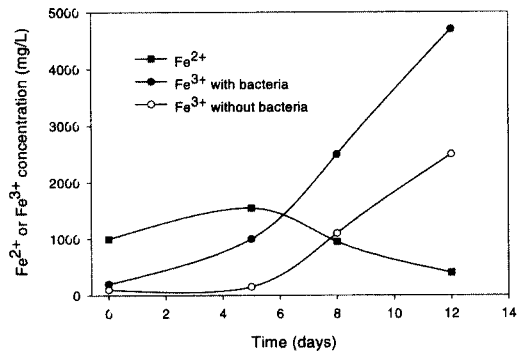

Figure 3. Variation of Fe^{2+} and Fe^{3+} concentrations under condition with or without *Acidithiobacillus ferrooxidans* at pH 2.2.

tration of Fe^{3+} significantly increased during reaction time in the presence of *At. ferrooxidans* over the extent of abiotic Fe^{3+} production. The result indicated that the bacteria facilitated the oxidation of Fe^{2+}. Such enrichment of a strong oxidizer, Fe^{3+}, in the solution can admittedly enhance the rate and extent of sulfide mineral dissolution and subsequent metal leaching.

Cu, Zn, and Cd were selected for solubilization experiments because the amounts of Cu and Zn in the dewatered sludge were higher than any other metal and because Cd is classified as a priority pollutant even in trace quantity. To investigate the relationship between the bioleaching efficiency and initial input amount of the dewatered sludge, different sludge content such as 1, 4, 10, and 15% (w/v) was added and leached. Increase in the sludge content resulted in a decrease of metal solubilization efficiency (Figure 4(a)). Such decrease in the metal extraction according to increase of the sludge content was likely due to buffering capacity of dewatered sludges.[14] Buffering agents of the sludge might cause consumption of H^+ which was produced by the cells. Since pH plays an important role in metal extraction, such inhibition against low pH maintenance in the solution likely retarded the rate of metal leaching.

In comparison, higher initial Fe^{2+} concentration, sulfur addition and fine particle size of the sludge did not enhance the metal solubilization over a period of 4 days (Figure 4(b) ~(d)). Increase in the initial Fe^{2+} addition did not result in an enhancement of metal solubilization (Figure 4(b)). At high substrate (Fe^{2+}) concentrations, the amount of enzyme is less than the amount of the substrate, and a second substrate molecule binds to the enzyme-substrate complex and forms an unreactive intermediate.[15] As well, the precipitation of iron precipitates such as jarosite might block pathways of the leaching solution into the solid heap and subsequently reduce the amount of dissolved metals.[16]

The fact that *At. ferrooxidans* used was not adapted for sulfur prior to the experiments can explain why sulfur addition did not enhance the bacterial activity, though sulfur is known as another energy source for the bacterium (Figure 4(c)). In this case, sulfur of additional quantity might restrict mass transfer of gas phase (e.g., O_2 and CO_2) and the leaching solution and retard the rate of bioleaching.

Such results can allow practitioners to reduce the cost of bioleaching of the sludge using *At. ferrooxidans* since the expense for providing additional Fe^{2+} and sulfur and for grinding the sludge can be reduced. If more extended bioleaching period is needed, however, proper addition of Fe^{2+} or sulfur as an energy source might be required.

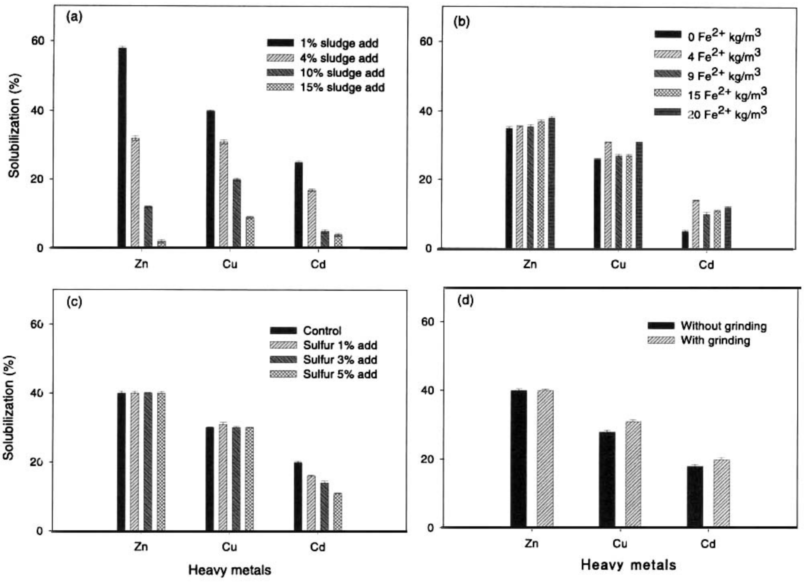

Figure 4. Effect of (a) sludge content, (b) ferrous iron concentration, (c) sulfur content and (d) grinding of dewatered sludge on the solubilization efficiency of heavy metals during 4 days of bioleaching period.

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Long-Term Bioleaching of the Dewatered Sludge

After a series of short-term bioleaching of the dewatered sludge, more experimentation was carried out with a larger reactor (reactor volume: 5 L and working volume: 2.5 L). The conditional parameters employed were based on the data received from the short-term bioleaching experiments. Figure 5 shows that the solubilization of the metals from the dewatered sludge tended to increase over time though 90% of this removal occurred within the first 10 days. The amounts of extracted Zn, Cu and Cd after 40 days of bioleaching are 41, 38, and 10%, respectively. Since pH is the most important factor in extracting metals from solid substrates, initial pH of 2.0 might significantly influence metal leaching observed during the initial period. Additionally, long-term maintenance of strong acidic environment which was attributed to bacterially-produced proton apparently increased metal extraction further.

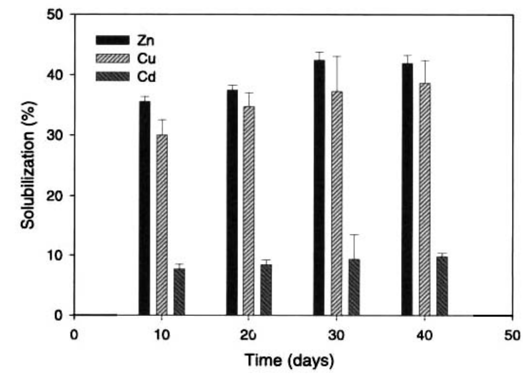

Figure 5. Solubilization of Zn, Cu and Cd by *Acidithiobacillus ferrooxidans* in the dewatered sludge over a period of 40 days.

Figure 6 presents SEM pictures of the dewatered sludge particles before and after bioleaching. Under the same magnification (× 1,000), it was observed that the particles of the dewatered sludge became smaller and initial morphology was not preserved due to active

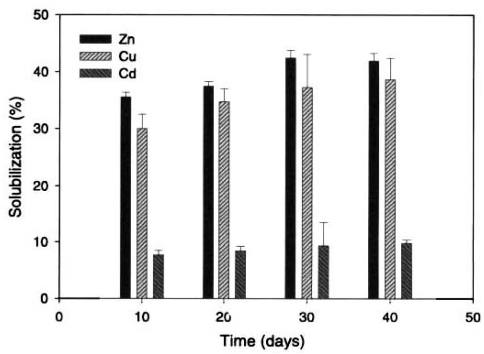

Figure 6. Pictures of scanning electron microscope (1,000-fold magnification) for the dewatered sludge particles before (0 day: left) and after (40 day: right) bioleaching using *Acidithiobacillus ferrooxidans*.

dissolution after 40 days of bioleaching. The following degradation processes may be assumed; (1) *At. ferrooxidans* adhered to the surface of dewatered sludge, (2) leaching by *At. ferrooxidans* took place, (3) upon continued leaching, cracks and pits on the surface of the dewatered sludge increased in size and (4) eventually, cracks and pits merged so that the particles were totally destroyed.

CONCLUSIONS

1. The heavy metals are closely associated with easily leachable phases such as exchangeable and carbonate fractions in the dewatered sludge.
2. The values of pH and ORP over the period of exponential cell growth were 2.0~2.5 and 500~600 mV, respectively.
3. As a result of short-term bioleaching, the initial sludge content affected the metal solubilization with the minimum sludge content showing the maximum leaching efficiency. However, higher initial Fe^{2+} concentration, sulfur addition and fine particle size of the sludge did not enhance the metal solubilization over a period of 4 days.
4. After 40 days of long-term bioleaching with the optimized conditions for the metal solubilization by *Acidithiobacillus ferrooxidans*, the extraction efficiency of Zn, Cu, and Cd reached 41, 38, and 10%, respectively. The result indicated that bioleaching using this acidophile can be effectively employed in the process of decontamination of heavy metal-laden dewatered sludge.

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